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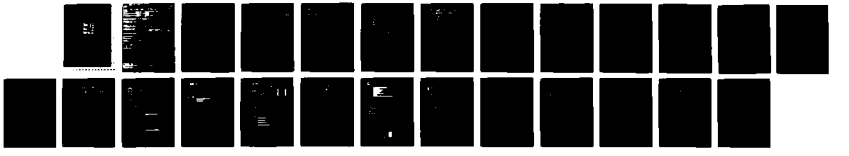

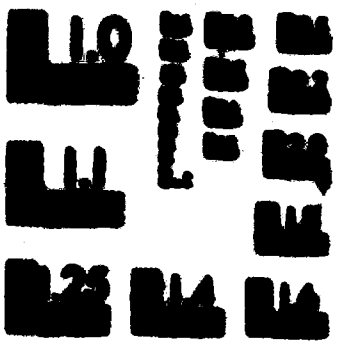

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Department of Medicine
Molecular Genetics and
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University of Massachusetts Medical Center
Worcester, MA 01605

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(U) Human Immune Responses to Dengue Viruses

Ennis, Francis A.

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We have analyzed the interaction between the peripheral blood lymphocytes (PBL) of non-immune donors and dengue virus-infected cells, which results in interferon (IFN) production. Autologous monocytes or the Epstein-Barr virus transformed B lymphoblastoid cell line (Raji cells) infected with dengue virus were used as IFN inducer cells. PBL produced IFN when cultured with dengue virus-infected cells. IFN was detected as early as 2 or 4 hours after exposure of PBL to dengue virus-infected cells and the titer reached maximum levels by 16 hours of incubation. Dengue virus-infected cells treated with glutaraldehyde or paraformaldehyde, which produced no infectious dengue virus, also induced IFN. These results indicate that PBL produced IFN in response to dengue virus-infected cells and that the production of IFN by PBL is due to stimulation of PBL by dengue virus-infected cells. The ability of dengue-infected cells to induce IFN correlated with the appearance of dengue antigens in infected cells. Characterization of IFN-producing PBL with monoclonal antibodies demonstrated that the IFN-producing cells were heterogeneous. The predominant IFN producing

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Abstract (cont'd)

cells were contained in HLA-DR+, M1+ and T3- subsets. The Leu11+ subset and Leu12+ subsets also contained some IFN-producing cells. The IFNs that were produced by the PBL exposed to dengue virus-infected cells were analyzed by radioimmunoassay employing monoclonal antibodies to detect specifically IFN-α or IFN-γ. IFNαwas the predominant IFN produced. In addition, dengue-infected monocytes induced low titers of IFNγ in some experiments, and when dengue virus-infected Raji cells as inducer cells, INFγ was detected in all the experiments. To determine whether the levels of IFN which were detected could prevent dengue virus infection, monocytes were treated with 400 IU/ml of IFNα before infection. Treatment of monocytes with IFN decreased the yield of infectious virus more than 99% and the percentage of dengue-antigen positive cells by 98%. These results suggest that IFNs produced by PBL in response to dengue virus-infected cells may have an important role in controlling dengue infection and in the pathogenesis of dengue hemorrhagic fever and shock syndrome.

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HUMAN IMMUNE RESPONSES TO DENGUE VIRUSES

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ANNUAL REPORT

FRANCIS A. ENNIS

JULY 1, 1986

Supported by

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Department of Medicine
Molecular Genetics and Microbiology
University of Massachusetts Medical Center
Worcester, Massachusetts 01605

SUMMARY

We have analyzed the interaction between the peripheral blood lymphocytes (PBL) of non-immune donors and dengue virus-infected cells, which results in interferon (IFN) production. Autologous monocytes or the Epstein-Barr virus transformed B lymphoblastoid cell line (Raji cells) infected with dengue virus were used as IFN inducer cells. PBL produced IFN when cultured with dengue virus-infected cells. IFN was detected as early as 2 or 4 hours after exposure of PBL to dengue virus-infected cells, and the titer reached maximum levels by 16 hours of incubation. Dengue virus-infected cells treated with glutaraldehyde or paraformaldehyde, which produced no infectious dengue virus, also induced IFN. These results indicate that PBL produced IFN in response to dengue virus-infected cells and that the production of IFN by PBL is due to stimulation of PBL by dengue virus-infected cells. The ability of dengue-infected cells to induce IFN correlated with the appearance of dengue antigens in infected cells. Characterization of IFN-producing PBL with monoclonal antibodies demonstrated that the IFN-producing cells were heterogeneous. The predominant IFN producing cells were contained in HLA-DR+, M1+ and T3- subsets. The Leu11+ subset and Leu12+ subsets also contained some IFN-producing cells. The IFNs that were produced by the PBL exposed to dengue virus-infected cells were analyzed by radioimmunoassay employing monoclonal antibodies to detect specifically IFN-α or IFN-γ. IFNα was the predominant IFN produced. In addition, dengue-infected monocytes induced low titers of IFNγ in some experiments, and when dengue virus-infected Raji cells as inducer cells, IFNγ was detected in all the experiments. To determine whether the levels of IFN which were detected could prevent dengue virus infection, monocytes were treated with 400 IU/mL of IFNα before infection. Treatment of monocytes with IFN decreased the yield of infectious virus more than 99% and the percentage of dengue-antigen positive cells by 98%. These results suggest that IFNs produced by PBL in response to dengue virus-infected cells may have an important role in controlling dengue infection and in the pathogenesis of dengue hemorrhagic fever and shock syndrome.

FOREWORD

This year of the research contract began on September 1, 1985.

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I. Introduction

The purpose of this study is to define the immune responses of humans to dengue viruses. These studies should provide data which will be helpful in understanding the complex immune responses to dengue infections. An improved understanding of immune responses to dengue virus is important in attempts to prevent disease by successful immunization.

Dengue virus infection is a major health problem in tropical and sub-tropical areas because of its severe complicatons, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (1). Primary dengue virus infection generally occurs without these complications (1). DHF/DSS are more often observed in patients suffering from secondary dengue virus infection with another serotype of dengue virus than that which caused the primary infection (2). Anti-dengue virus antibodies which can enhance dengue virus infection of Fc receptor bearing cells have been thought to play an important role in the pathogenesis of DHF/DSS (2,3). It has been postulated that these enhancing antibodies increase the number of infected monocytes, which are the major source of virus production, and that the immune-mediated destruction of the dengue virus-infected monocytes leads to the complications (2,4). The immune mechanisms which are responsible for the destruction of dengue virus-infected monocytes have not been defined. Effector mechanisms which should be considered include natural killer (NK) cell-mediated lysis, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent complement-mediated lysis, and cytotoxic T lymphocytes.

We recently reported that dengue virus-infected cells were lysed by non-immune human lymphocytes to a greater degree than uninfected cells in natural killer (NK) assays (5). The effector cells were characterized as two subsets. The predominant effector cells were Leu 11^+ cells which also lysed K562 cells very well. $T3^+$ cells which do not lyse K562 cells also lysed dengue-infected cells to lesser degree. The mechanism of preferential lysis of dengue virus-infected cells is not known. We detected high titers of IFN activity in the supernatants of the NK assays (5). However, augmented lysis of dengue virus-infected cells by non-immune PBL may not be due to the IFN produced, because anti-IFN antiserum which neutralized all the detectable levels of IFN did not inhibit the lysis of dengue-infected cells.

IFNs appear to have very important roles in the host response to virus infections because they have potent antiviral activities and modulate immune responses (6). IFNs augment natural killer (NK) activity (7), increase the expression of major histocompatibility antigens (8), and increase the susceptibility of virus-infected cells to lysis by virus-specific cytotoxic T lymphocytes (9).

In the present report we analyze in more detail the interaction between the PBL of non-immune donors and dengue virus-infected cells, which results in IFN production. We use dengue virus-infected B lymphoblastoid cell line (Raji cells) and dengue virus-infected autologous monocytes as inducer cells. We identify the IFN producer cells using monoclonal antibodies and employ radio-immunoassay (RIA) with monoclonal antibodies to specifically detect human IFNα and IFNγ (10).

II-A. IFN-induction from non-immune PBL by dengue virus-infected Raji cells.

(1) Induction of IFN by dengue virus-infected cells

When the PBL of non-immune donors were cultured with dengue virus-infected Raji cells, IFN activity was detected at titers from 150 to 1600 U/mL in the supernatant fluids. Very low titers of IFN were detected with PBL were cultured alone or with uninfected cells (Table 1). IFN was not detectable in the culture supernatants of dengue virus type 2-infected cells alone or uninfected cells alone. We also examined IFN production using cells infected with dengue virus types 1, 3 and 4 as inducer cells. Cells infected with these other types of dengue virus induced high titers of IFN similar to those induced by dengue virus type 2-infected cells (Table 2).

Table 1

PRODUCTION OF IFN BY PBL IN RESPONSE TO DENGUE VIRUS-INFECTED CELLS

Donor	IFN (units/mL)*		
	Infected** Inducer Cells	Uninfected** Inducer Cells	No Inducer Cells
A	800	<12	<12
B	400	75	75
C	400	25	<25
E	800	25	<12
F	1600	50	<6
G	200	25	<25
H	800	50	25
K	1600	100	50
L	600	25	<12
P	800	<25	<25
Q	150	50	<25
R	800	12	<12
S	800	25	<25

*IFN was assessed by bioassay after 16 hours of incubation. The ratio of PBL:inducer cell was 50:1.

**Significance was determined by paired t test between the titers of induced by dengue-infected inducer cells and those induced by uninfected inducer cells. Statistically significant; $p<0.001$.

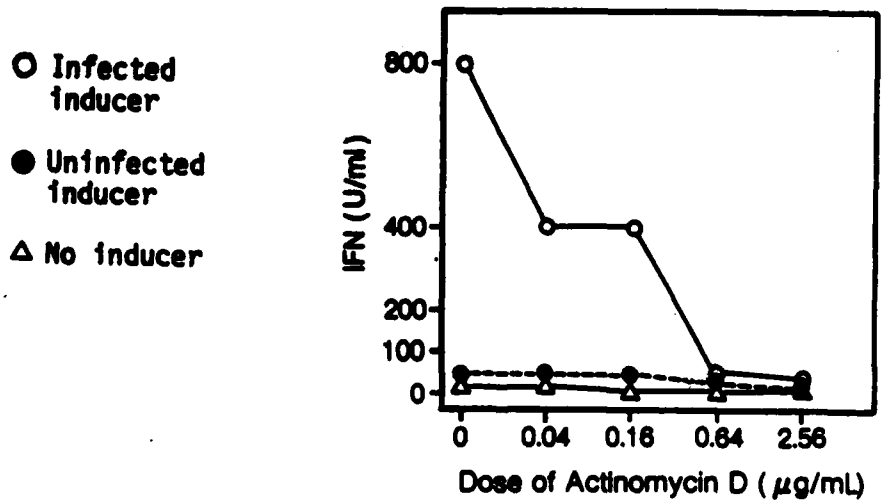
Table 2

INDUCTION OF IFN BY RAJI CELLS INFECTED WITH DENGUE VIRUS TYPES 1, 2, 3 OR 4

Donor	INTERFERON (Units/mL) Dengue 1 Raji	Dengue 2 Raji	Dengue 3 Raji	Dengue 4 Raji	Uninfected Raji	No Inducer Cells
H	300	400	600	1200	<25	<25
L	400	600	800	800	25	<12
P	400	800	800	600	<25	<25
R	600	800	1200	400	12	<12
S	200	800	400	600	25	<25
T	300	150	300	400	50	<25

In order to eliminate the possibility that dengue virus-infected cells produced IFN after exposure to PBL, PBL and dengue virus-infected cells were pretreated with actinomycin D at the dose of 0.04 to 2.50 µg/mL. Pretreatment of PBL with actinomycin D did not change the viability of the PBL, the dengue virus-infected or the uninfected cells. Pretreatment of PBL with actinomycin D at concentrations of 0.64 and 2.56 µg/mL decreased the production of IFN from 800 to 25 U/mL; however, pretreatment of dengue virus-infected cells did not change the titer of the produced IFN (Figure 1). These results indicate that PBL produce the IFN, but not the dengue virus-infected cells.

Figure 1. Effect of actinomycin D treatment of PBL on IFN production.



(2) Kinetics and effects of cell concentration of IFN production

The time course of IFN production was evaluated. IFN was detected as early as 2 hours after the beginning of incubation. The titer reached the maximum at 16 hours of incubation (Figure 2). The dose-response effect of various numbers of inducer cells was then evaluated. 5 x 10^5 PBL were cultured with 10^1 to 10^6 dengue virus-infected cells for 15 hours (Figure 3). There was an obvious dose-response relationship between the number of inducer cells (from 10^1 to 10^5) and the titer of IFN induced. 10^6 inducer cells were half as effective as 10^5 inducer cells, which may have been due to decreased contact between PBL and inducer cells at such a high concentration of cells.

Figure 2. Time course of IFN induction.

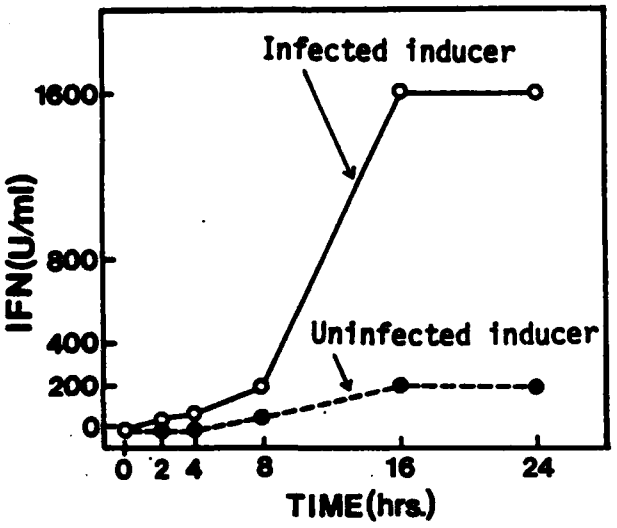
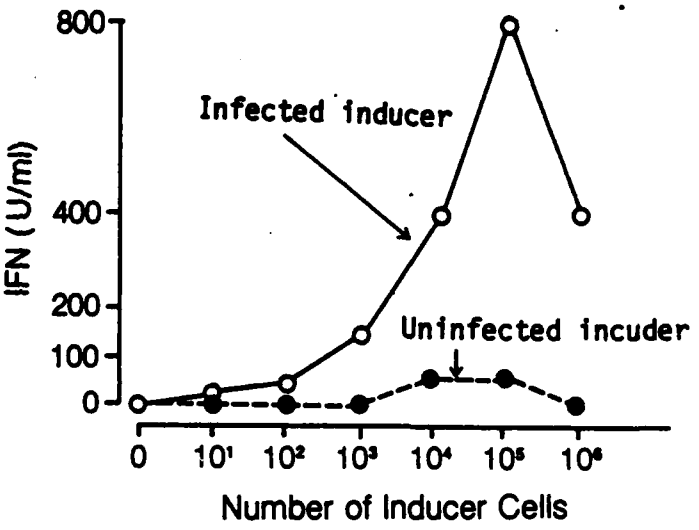


Figure 3. Dose response study of PBL-inducer cells.



(3) Induction of IFN by glutaraldehyde-treated, dengue virus-infected cells

Dengue virus-infected Raji cells produced a low titer of infectious dengue virus (230 PFU/ml after 16 hours of incubation of 5 x 10^4 cells/mL). This observation raised the possibility that the IFN may have been produced by infection of PBL with dengue virus and not by the stimulation of PBL by dengue virus-infected cells, so we performed additional studies.

We therefore examined glutaraldehyde-treated, dengue virus-infected cells for their ability to induce IFN. The results in Table 3 shows that the glutaraldehyde-treated cells also induced IFN from PBL. These glutaraldehyde-treated, dengue virus-infected cells did not produce infectious virus during 24 hours of cultivation. In addition, a dengue virus-infected cells line, which produces no infectious virus, also induced a high titer of IFN. These results indicate that production of IFN by non-immune PBL is due to stimulation of PBL by dengue virus-infected cells and is not secondary to infectious dengue virus.

Table 3

INDUCTION OF IFN BY GLUTARALDEHYDE-TREATED, DENGUE VIRUS-INFECTED CELLS

INDUCER*	TREATMENT** WITH GLUTARALDEHYDE	IFN (UNITS/ML)*** DONOR U	DONOR R
Infected	-	800	400
Infected	+	300	100
Uninfected	-	38	<25
Uninfected	+	<25	<25

*PBL were cultured with glutaraldehyde-treated or untreated Raji cells. The ratio of PBL:inducer cells was 40:1.
**Glutaraldehyde treatment resulted in the loss of viability of 100% of inducer cells as detected by trypan blue testing.
***IFN was assessed by bioassay after 16 hours of incubation.

(4) Characterization of IFN-producing PBL using monoclonal antibodies

PBL which produce IFN in response to dengue virus-infected cells were characterized using three monoclonal antibodies; OKM1, anti-Leu11, and OKT3 antibodies. The PBL were pretreated with monoclonal antibody and complement, and incubated with inducer cells for 16 hours (Table 4). After this treatment, less than 2% of the residual cells reacted with the monoclonal antibody which had been added with complement. IFN activity was assessed by bioassay. Pretreatment of PBL with OKM1 and complement, and anti-Leu11 antibody and complement decreased the titer of the IFN produced in all experiments. Low but significant titers of IFN were, however, produced by PBL depleted of $M1^+$ cells or $Leu11^+$ cells. Pretreatment of PBL with OKT3 and complement did not decrease the titer of IFN produced.

Table 4

IFN PRODUCTION BY PBL PRETREATED WITH MONOCLONAL ANTIBODIES AND COMPLEMENT

Treatment of effector cells with C' and antibody to	IFN (Units/mL)* Donor K		Donor E	
	Infected	Uninfected	Infected	Uninfected
-	400	<12	400	6
M1	25	<12	50	<6
Leu 11	25	<12	100	<6
T3	600	<12	400	<6

In the next series of experiments PBL were sorted by FACS after reaction with OKM1, anti-Leu11, or OKT3 antibodies, and were then incubated with inducer cells (Table 5). The purity of each of the sorted cell populations was more than 96%. $M1^+$ and $T3^-$ cells produced higher titers of IFN than did $M1^-$ and $T3^+$ cells, respectively. $Leu11^+$ cells and $Leu11^-$ cells produced almost the same titer of IFN. The results shown in Tables 4 and 5 are consistent and indicate that the predominant IFN-producing cells are contained in $M1^+$ and $T3^-$subsets, and that the $Leu11^+$ subset contains some IFN-producing cells.

Table 5

PRODUCTION OF IFN BY PBL AFTER SORTING WITH OKM1 ANTI-LEU11, OR OKT3 ANTIBODY

Samples from Donor		Effector Cells	IFN (Units/mL)* Infected Inducer Cells	Uninfected Inducer Cells	No Inducer Cells
Exp. 1	E	M1+	300	50	<12
		M1-	12	<12	<12
	H	M1+	400	50	25
		M1-	50	<6	<6
Exp. 2	H	Leu 11+	200	<12	<12
		Leu 11-	100	<12	<12
	K	Leu 11+	100	18	<6
		Leu 11-	50	<6	<6
Exp. 3	H	T3+	<25	<12	<12
		T3-	200	<12	<12
	K	T3+	12	<3	<3
		T3-	200	<3	<3

*IFN was assessed by bioassay after 16 hours of incubation. Ratio of PBL:inducer cell was 30:1.

(5) Characterization of produced IFN

The IFNs produced were characterized by radioimmuno and bioassays. In the bioassay the produced IFN was characterized by IFNα, because it was neutralized by antiserum to IFNα but was not significantly neutralized by antisera to IFNβ and IFNγ (data not presented). In the radioimmunoassay, however, some IFNγ as well as IFNα was detected in the supernatant fluids obtained from cultures which contained both PBL and dengue virus-infected cells. Although the titer of IFNγ was not as high as that of IFNα, the presence of IFNγ was consistent in all experiments (Table 6).

Table 6

CHARACTERIZATION BY RADIOIMMUNOASSAY (RIA) OF IFNs INDUCED BY DENGUE VIRUS-INFECTED CELLS

PBL from Donor	IFN (Units/mL)* Infected** Bio-Assay	Infected** RIA*** IFNα†	Infected** RIA*** IFNγ§	Uninfected** Bio-Assay	Uninfected** RIA IFNα†	Uninfected** RIA IFNγ§	No inducer Bio-Assay	No inducer RIA IFNα	No inducer RIA IFNγ
H	1280	208	67	<10	<10	17	<10	<10	9
P	1580	261	30	<10	<10	3	<10	<10	1
R	2000	520	27	<10	<10	7	<10	<10	<1

*IFN was assessed by radioimmuno and bioassays after 16 hours of incubation. Ratio of PBL:inducer cell was 40:1.

**Significance was determined by paired t tests between the titers of IFNs induced by dengue-infected inducer cells and those induced by uninfected inducer cells. Differences were measured comparing the amount of IFN induced by dengue-infected to uninfected inducer cells. Statistically significant;†$p<0.02$,§$p<0.05$.

***Radioimmunoassay was performed using monoclonal antibodies specific to IFNα and IFNγ as described previously (10).

We then characterized the PBL which produce IFNα and IFNγ using OKM1 and OKT3 antibodies, because these two antibodies most clearly discriminated IFN-producing PBL as shown in Tables 4 and 5. Treatment of PBL and OKM1 and complement decreased the production of both IFNα and IFNγ as measured by RIA (Table 7). PBL pretreated with OKT3 and complement (T3$^-$ cells) produced a higher titer of IFNα and IFNγ than did PBL pretreated with complement alone, probably because of the resulting enrichment of IFN-producing cells. The results indicate that both IFNα and IFNγ are predominantly produced by PBL contained in M1$^+$ and T3$^-$ subsets.

Table 7

CHARACTERIZATION OF PBL WHICH PRODUCE IFNα AND IFNγ USING OKM1 AND OKT3 ANTIBODIES

Treatment of Effector	IFN (UNITS/ML) detected by RIA					
	IFNα			IFNγ		
	Infected Inducer	Uninfected Inducer	No Inducer	Infected Inducer	Uninfected Inducer	No Inducer
Exp. 1 (Donor R)						
C'	270	<10	<10	23	<5	<5
OKM1+C'	100	<10	<10	<5	<5	<5
OKT3+C'	340	<10	<10	25	<5	<5
Exp. 2 (Donor M)						
C'	330	<10	<10			
OKM1+C'	96	<10	<10			
OKT3+C'	640	<10	<10			
Exp. 3 (Donor P)						
C'				15	5	<1
OKM1+C'				4.5	<1	<1
OKM3+C'				50	7.5	<1

*IFN was assessed by RIA on samples of supernatants removed after 16 hours of incubation. Ratio of PBL:inducer cell was 40:1.

IIB. IFN-induction from non-immune PBL by dengue virus-infected, autologous monocytes.

We then analyzed IFN induction using autologous dengue virus-infected monocytes as inducers because this autologous system may reflect in vivo phenomena better than the allogeneic system, when dengue-infected Raji cells were used as inducers. Adherent cells were enriched from peripheral blood mononuclear cells by adherence to plastic petri dishes. These adherent cells were then considered to be monocytes. The monocytes were infected with dengue virus type 2 at a multiplicity of infection of 2 PFU per cell in a presence of anti-dengue antiserum diluted 1:2 x 10^4, and cultured at 37°C for 1 or 2 days and then were used as IFN-inducer cells.

(1) IFN-induction from non-immune PBL by dengue-infected autologous monocytes

When dengue non-immune PBL were cultured with dengue-infected, autologous monocytes for 20 hours, high titers of IFN activity was detected in the supernatants. Low titers of IFN activity were detected in the supernatants of PBL cultured alone, or of dengue-virus infected monocytes cultured alone. When PBL were cultured with uninfected monocytes, very low titers of IFN activity was detected (Figure 4). In order to confirm that PBL produced IFN in response to dengue infected-monocytes, we treated PBL with actinomycin D at a dose of 0.64 ug/mL. Pretreatment of PBL with actinomycin D decreased the production of IFN from 800 units to 50 units/mL (Figure 5). These results indicate that PBL produce high titer of IFN in response to dengue-infected monocytes.

Figure 4 Dengue-infected Monocytes induce IFN from non-immune autologous PBL

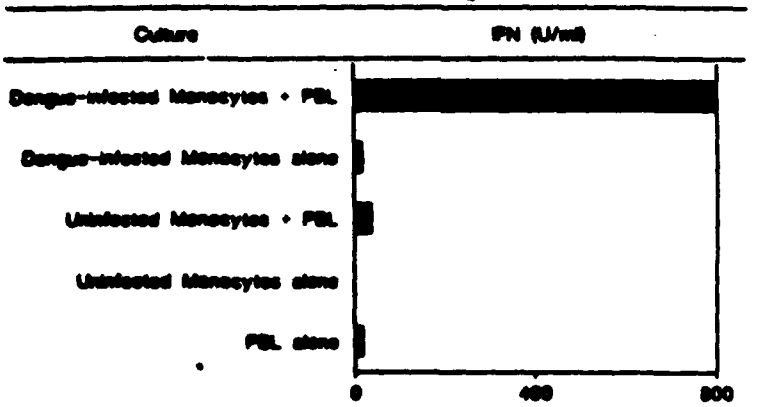
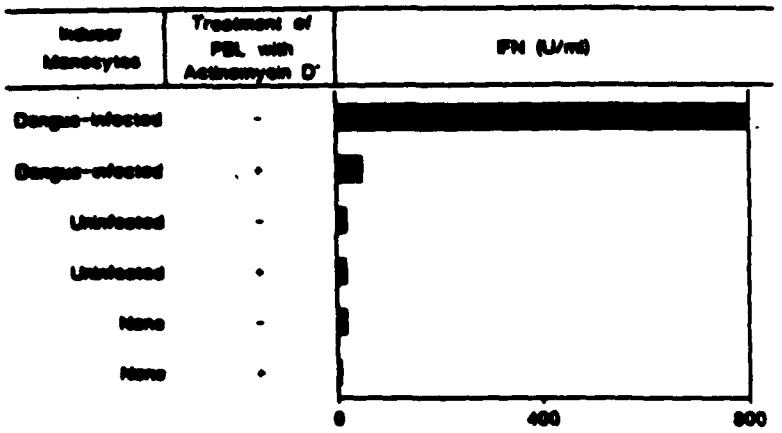


Figure 5 IFN is produced by PBL, not by Monocytes

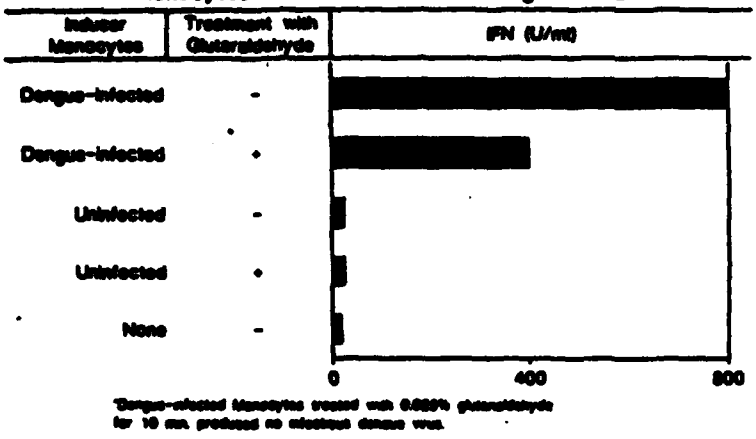


*PBL were pretreated with 0.64 µg of Actinomycin D/ml for 1 hour

(2) Induction of IFN by glutaraldehyde-treated dengue virus-infected autologous cells.

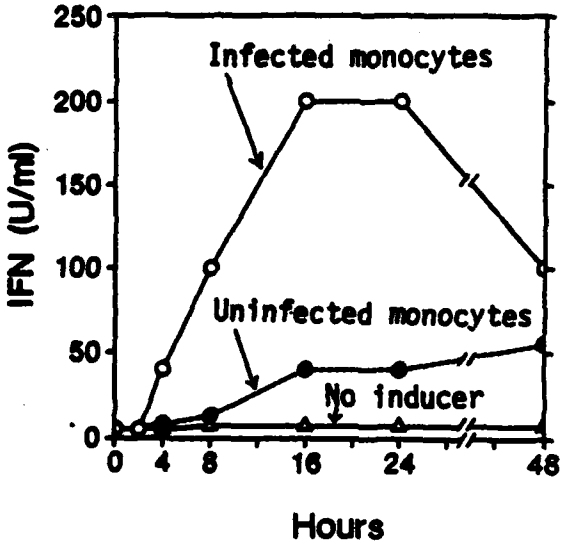
We examined glutaraldehyde-treated, dengue virus-infected cells for their ability to induce IFN. The glutaraldehyde-treated dengue virus-infected, autologous monocytes, which did not produce infectious dengue virus also induced IFN from PBL (Figure 6). A time course study showed that IFN was detected as early as 4 hours after the beginning of incubation, and reached the maximum by 16 hours of incubation (Figure 7).

Figure 6. Glutaraldehyde-treated Dengue-infected Monocytes* induce IFN from autologous PBL



*Dengue-infected Monocytes treated with 0.025% glutaraldehyde for 10 min. produced no infectious dengue virus.

Figure 7. Time course of IFN induction from PBL by dengue-infected monocytes.



The dose-response effect of various numbers of inducer cells was then evaluated. There was an obvious dose-response relationship between the number of inducer cells and the titer of IFN induced (Figure 8). The ability of dengue-infected cells to induce IFN was then examined at various times after infection. The infected monocytes were able to induce IFN 8 hours after infection, a time when dengue antigen-positive cells become detectable. The ability of dengue-infected cells to induce IFN reached a maximum at 24 hours after infection when the percentage of antigen-positive cells also reached a maximum (Figure 9).

Figure 8. Dose response study of PBL and dengue-infected monocytes.

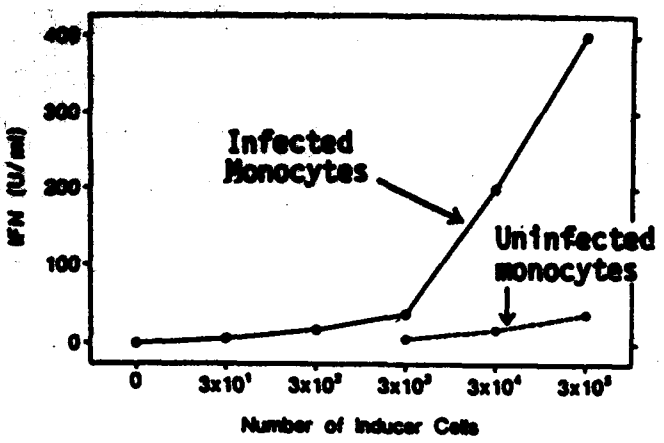
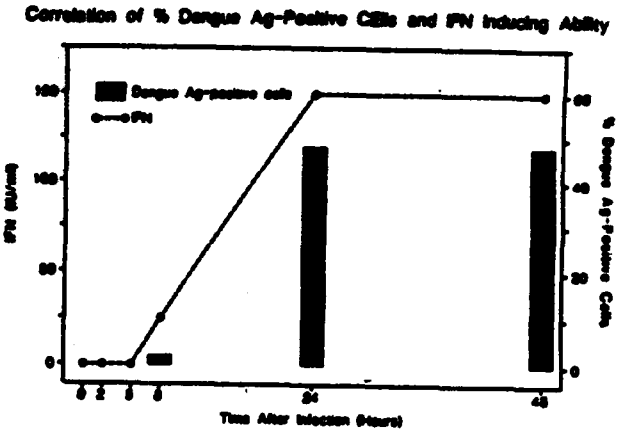


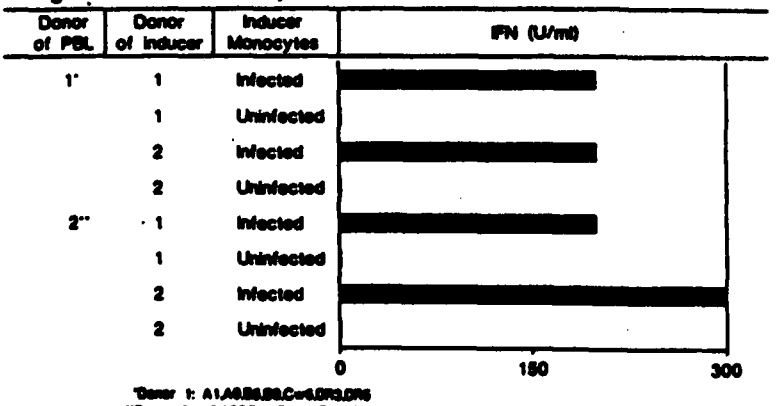
Figure 9.



(3) MHC-compatibility is not required for the induction of IFN

To learn whether IFN-induction requires the MHC-compatibility, we analyzed IFN induction using the PBL of two donors who have completely different MHC antigens. The dengue-infected monocytes of donors 1 and 2 induce similar titers of IFN from the PBL of donor 1. The monocytes of donors 1 and 2 also induced similar titers of IFN from PBL of donor 2. These results indicate that MHC compatibility is not required in IFN-induction from PBL by dengue-infected monocytes (Figure 10).

Figure 10 IFN induction from PBL by Dengue-infected Monocytes is not MHC-restricted



*Donor 1: A1,A68,B8,Cw4,DR3,DR5
**Donor 2: A2,A68,B35,Bw60,Cw3,Cw4,DR4

(4) Characterization of produced IFN

The IFN produced were characterized by radioimmunoassay (RIA). The predominant IFN induced from PBL by dengue-infected monocytes was IFNα, however, in some experiments low titers of IFNγ were also detected in the supernatants as shown in Table 8.

Table 8

CHARACTERIZATION OF RIA OF IFNS INDUCED BY DENGUE VIRUS-INFECTED, AUTOLOGOUS MONOCYTES

Culture	IFN (units/mL) determined by RIA IFNα	IFNγ	IFN (units/mL) determined by bioassay
PBL + Infected Monocytes	250	4	1400
Infected Monocytes alone	<3	<1	<10
PBL + Uninfected Monocytes	<3	<1	<10
Uninfected Monocytes alone	<3	<1	<10
PBL alone	<3	<1	<10

(5) Characterization of IFN producing PBL using monoclonal antibodies

PBL which produce IFN in response to autologous dengue-infected cells were characterized using 5 monoclonal antibodies; OKT3, anti-Leu11, anti-Leu12, OKM1 and anti-HLA DR antibodies. Table 5 shows the monoclonal antibodies and their specificities.

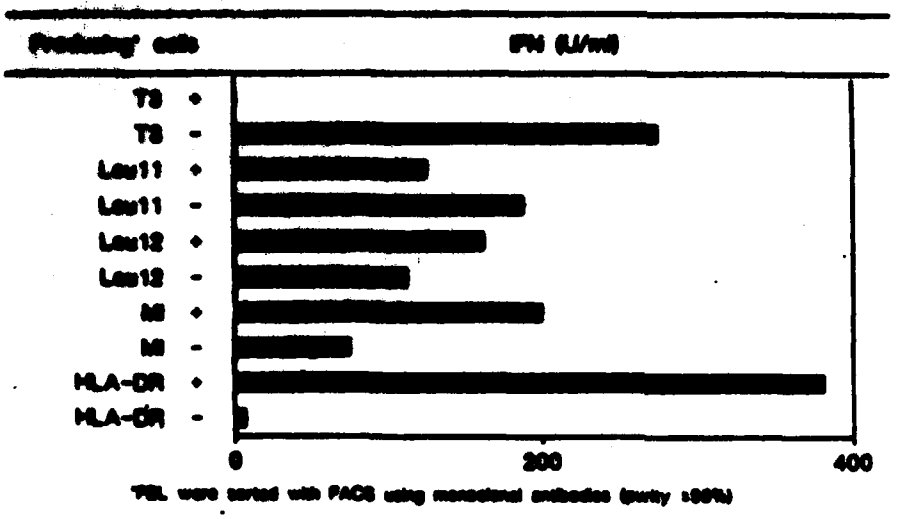
Table 9

MONOCLONAL ANTIBODIES USED FOR CHARACTERIZATION OF IFN-PRODUCING PBL

Monoclonal Antibody	Specificity
OKT 3	T cells
Anti-Leu11	NK cells
Anti-Leu12	B cells
OKM1	Monocytes, NK cells
Anti-HLA-DR	Monocytes, B cells, Activated T cells

PBL were sorted by FACS after reaction with these monoclonal antibodies, and were then incubated with inducer cells (Figure 11). HLA-DR$^+$ cells and T3$^-$ cells exclusively produced IFN. Neither HLA-DR$^-$ cells nor T3$^+$ cells produced IFN activity. Leu11$^+$ cells and Leu11$^-$ cells Leu12$^+$ cells and Leu12$^-$ cells produced almost the same titers of IFN. M1$^+$ cells produced higher titers of IFN than M1$^-$ cells. These results indicate that IFN-producing cells are heterogeneous, and that they can be characterized as HLA-DR$^+$, non-T cells.

Figure 11. . Heterogeneous PBL produce IFN



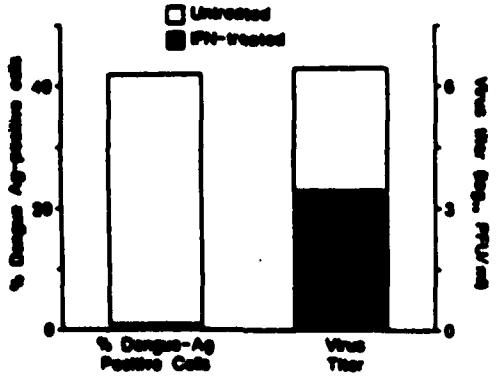
PBL were sorted with FACS using monoclonal antibodies (purity >90%)

II-C. Inhibition of dengue virus infection by IFN

We showed in the above sections that autologous dengue virus-infected cells induce IFN from non-immune PBL. The induced IFN are primarily IFNα. We then analyzed whether the IFN produced are effective in prevention of dengue virus infection.

Human monocytes were cultured with human IFNα at a concentration of 400 units/mL for 18 hours, and then infected with dengue 2 virus at the m.o.i. of 2 in the presence of anti-dengue 2 antibody. About 40% of the monocytes which were not pretreated with IFN were infected as determined by indirect FA staining, and high titers of dengue virus were detected in the culture supernatant on day 2. Monocytes pretreated with IFN contained only 0.8% of antigen positive cells. Yield of the infectious dengue virus was reduced >99% below the levels of untreated cultures (Figure 12). These results indicate that the levels of IFN which were induced from PBL by dengue virus-infected cells can inhibit the further infection of human monocytes by dengue virus.

Figure 12. Effect of IFNα (400 units/mL) on dengue virus infection of human monocytes.



III. Discussion

We have described the results of experiments which indicate that dengue virus-infected cells induce IFN from non-immune PBL. Dengue virus-infected B lymphoblastoid cells (Raji) and dengue virus-infected, autologous monocytes induced similar levels of IFN from PBL. The IFN-induction is not due to the infection of PBL by infectious dengue virus, but rather it appears to be a result of PBL stimulation by the dengue-infected cells.

Production of IFN by the PBL of non-immune donors in response to various virus-infected cells has been reported (11-13). Most investigators have characterized the produced IFN as IFNα. We detected high levels of IFNα and low levels of IFNγ in the supernatant fluids of cultures containing PBL and dengue virus-infected cells. The present results are interesting and suggest that cells infected with other viruses may induce IFNγ from the PBL of non-immune donors which previously may not have been detected due to the inherent limitations of the bioassay and neutralization techniques. We had failed to detect IFNγby standard neutralization tests because the dilution techniques used in the neutralization assay would not detect a lower titered IFNγcomponent.

Lymphocytes which produced IFN after addition of virus have been characterized as null cells (14) and as large granular lymphocytes (15). Our results are somewhat consistent with the previous reports which indicate that IFN-producing cells have characteristics of NK cells, because it has been reported that most human NK cells have M1 antigen (16) and the Leu11 antigen is expressed on essentially all functional NK cells in peripheral blood (17). However, we also found that PBL contained exclusively in HLA-DR$^+$ fraction produce IFN and that Leu12$^+$ cells which are B cells produce some IFN. It has been reported that very few human NK cells have HLA-DR antigen. These results indicate that human PBL which produces interferon are heterogeneous.

The role of the IFNs produced by PBL exposed to dengue virus-infected cells in dengue infections remains to be studied. The present experiments demonstrate that the levels of IFN induced from non-immune PBL by dengue infected monocytes limited the spread of dengue infection. It has been reported that mouse serum which contained type 1 IFN activity showed therapeutic effects against dengue virus infection in mice (18). IFNγ has been reported to be more active as an immunoregulatory agent than IFNα and IFNβ (8). IFNγ but not IFNα and IFNβ induced HLA-DR antigen on human monocytes, and IFNγ was more ative in inducing HLA-A and B antigens than IFNα and IFNβ (19). Although the titer of IFNγ produced was not as high as that of IFNα, it is possible that IFNγas well as IFNαmay play a role in recovery from dengue virus infection or in the pathogenesis of dengue hemorrhagic fever and dengue shock syndrome.

The mechanism of IFN-induction by dengue virus-infected cells remains to be elucidated. Glutaraldehyde-treated, dengue-infected cells which do not produce infectious dengue virus were also able to induce IFN. In addition, a dengue virus-infected Raji cell line which does not produce detectable infectious dengue virus induced IFN. These resuls indicate that infectivity of the dengue virus is not essential for the induction of IFN, and it is probable that some component expressed on the infected cells is responsible. Dengue

virus has 3 structural proteins; V1, V2 and V3. V3 may be the only exposed protein antigen on the virion and is responsible for hemagglutination (20). It has been reported that the hemagglutinin-neuraminidase glycoprotein of Sendai virus can induce IFN from mouse spleen cells (21). This suggests that dengue viral proteins expressed on infected cells may be responsible for the induction of IFN. It has also been reported that non-virion proteins are present on the surface of dengue virus-infected cells (22). Recently, it has been reported that monoclonal antibody to the NS1 antigen produced by infection with another flavivirus (Yellow Fever, strain 17D) fixed complement and lysed infected cells (23). Therefore, it is possible that nonvirion proteins may be rresponsible for inducing IFNs, but this requires further analysis. Thus, two interesting questions remain to be elucidated. i) Are proteins expressed on dengue virus-infected cells responsible for the induction of IFN? and ii) What role does the produced IFN play in the immune response to dengue virus? Answers to these questions will lead us to a better understanding of immune responses and their possible role in dengue virus infections.

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